promoter region, was amplified with the primers pSsrAHindIIIfw (5' TTC TAA AAG CTT AGT GCT TGA TTC GAA AAT CAG GCC TGT G 3') (SEQ ID NO:___) and pSsrADDintRV (5' GAG CTC GCT GCG CTT ATT AGT CGT CTA ATG CTA CGT TTT GGT TAA 3' (SEQ ID NO:___); contains the alteration of the two alanine codons in the SsrA tag sequence into codons for aspartic acid residues). In addition, an overlapping 3'end part of ssrA was amplified with the primers pSSrADDintFW (5' TTA ACC AAA ACG TAG CAT TAG ACG ACT AAT AAG CGC AGC GAG CTC 3' (SEQ ID NO:___); also containing the alteration of the two alanine codons into codons for two aspartic acid residues) and pSsrASphIRV (5' CCT CCG TGC ATG CTT CCT CTT ATT TAT TGA CAG AAA TCT G 3') (SEQ ID NO:___). Both fragments were assembled in a fusion PCR with primers pSsrAHindIIIFW and pSsrASphIRV, and cloned in pCR2.1-TOPO, resulting in plasmid pSsrADD. The correct sequence of the fusion product in pSsrADD was confirmed by DNA sequencing. Next, a selective marker (the Tc resistance cassette derived from pDG1515; Guérout-Fluery et al. 1995. Antibiotic-resistance cassettes for Bacillus subtilis. Gene 167:335-336) that functions in B. subtilis, was cloned into the EcoRV site of pSsrADD, resulting in plasmid pSsrADDTc. Finally, B. subtilis 168 IssrA^{DD} and WB600 IssrA^{DD} were obtained by a Campbell-type integration (single cross-over) of pSsrADDTc into one of the disrupted ssrA regions on the chromosome of B. subtilis 168 ΔssrA and WB600 ΔssrA, respectively. These strains contain an active copy of the ssrA^{DD} gene on the chromosome (under control of the native ssrA promoter) and a disrupted copy of wild-type ssrA (insertion of the Sp resistance marker), as confirmed by PCR. To construct B. subtilis WB600 ΔctpA, WB600 was transformed with chromosomal DNA of BSE-23. In BSE-23, the ctpA gene is replaced by a spectinomycin resistance cassette (Edwin Lee, Genencor International Palo Alto, unpublished). WB600 ΔyvjB was obtained as follows: yvjB and its flanking regions (approximately 3.5 kb) was amplified by PCR with the primers pYvjBFW (5' AGA GTT TTA AAT CTC TCG GGA GAA ACA

CAT GGA TGA CAT T 3') (SEQ ID NO:___) and pYvjBRV (5' TGT ATA TGT AAA TTT CAG ATC ATC ATA AAT ATC TGC TAT T 3') (SEQ ID NO:___) and cloned in pCR2.1-TOPO, resulting in plasmid pTPYvjB. Plasmid pTPYvjBTc was obtained by replacing an internal SmaI-AccI fragment of the yvjB gene in pTPYvjB with a pDG1515-derived Tc resistance marker (Guérout-Fluery et al. 1995. Antibiotic-resistance cassettes for Bacillus subtilis. Gene 167:335-336). Finally, B. subtilis WB600 ΔyvjB was obtained by a double cross-over recombination event between the disrupted yvjB gene of pTPYvjBTc and the chromosomal yvjB gene. To construct B. subtilis WB600 IclpP, the 5' end region of the clpP gene was amplified by PCR with the primers pClpPEcoFW (5' CTT ACC GAA TTC GTG AAG GAG GAG CAT TAT G 3') (SEQ ID NO:___) containing a EcoRI site, and pClpPBamRV (5' GCC TTT GGA TCC GGC TGC AAG CAG GAA CGC 3') (SEQ ID NO:___) containing a BamHI site. The amplified fragment was cleaved with EcoRI and BamHI, and cloned in the corresponding sites of pMutin2 (Vagner et al. 1998. A vector for systematic gene inactivation in Bacillus subtilis. Microbiology 144:3097-3104), resulting in plasmid pMutClpP. B. subtilis WB600 IclpP was obtained by a Campbell-type integration (single cross-over) of pMutClpP into the clpP region on the chromosome. Cells of this strain are depleted for ClpP by growing them in medium without IPTG (Vagner et al. 1998).

Table 1. Plasmids and Strains

Plasmid/Strain	Properties	Reference
pLATIL3	derivative of pGB/IL-322: contains the human *IL-3* gene fused to the sequence encoding the signal peptide of *B. licheniformis* α-amylase (*amyL-hIL-3*); the *amyL-hIL-3* gene fusion is under control of the amylase promoter; 4.3 kb; Nm^R	Van Leen et al. 1991. Production of human interleukin-3 using industrial microorganisms. Biotechnology **9**:47-52.
pLATIL3TERM	derivative of pLATIL3; contains the transcription terminator of the *B. subtilis folC* gene inserted just in front of the stop codon of *amyL-hIL-3*; 4.1 kb; Nm^R	This work

Plasmid/Strain	Properties	Reference
pLATIL3BStag	derivative of pLATIL3; contains *amyL-hIL-3* fused at the 3'end to the sequence encoding the *B. subtilis* SsrA peptide tag (AGKTNSFNQNVAL**AA**); 4.2 kb; Nm^R	This work
pLATIL3DDtag	derivative of pLATIL3; contains *amyL-hIL-3* fused at the 3'end to the sequence encoding a variant SsrA-DD-tag (AGKTNSFNQNVAL**DD**); 4.2 kb; Nm^R	This work
pLATIL3ECtag	derivative of pLATIL3; contains *amyL-hIL-3* fused at the 3'end to the sequence encoding the *E. coli* SsrA peptide tag (AANDENYALAA); 4.2 kb; Nm^R	This work
pCR2.1-TOPO	TA cloning vector for PCR products; 3.9 kb; Ap^R; Km^R	Invitrogen
pTPSsrA	pCR2.1-TOPO derivative; carrying the *ssrA* gene + flanking regions; 6.1 kb; Ap^R; Km^R	This work
pSsrASp	derivative of pTPSsrA for the disruption of *ssrA*; 7.0 kb; Ap^R; Km^R; Sp^R	This work
pSsrADD	pCR2.1-TOPO derivative; carrying a $ssrA^{DD}$ gene variant: the last two codons of the tag sequence in *ssrA* (gct gcc) encoding two alanines are changed into gac gac, encoding two aspartic acid residues; 4.6 kb; Ap^R; Km^R	This work
pSsrADDTc	derivative of pSsrADD; carrying $ssrA^{DD}$ and a Tc resistance cassette; for integration of $ssrA^{DD}$ on the *B. subtilis* chromosome; 6.8 kb; Ap^R; Km^R; Tc^R	This work
pTPYvjB	pCR2.1-TOPO derivative; carrying the *yvjB* gene + flanking regions; 7.4 kb; Ap^R; Km^R	This work
pTPYvjBTc	derivative of pTPYvjB for the disruption of *yvjB*; 8.9 kb; Ap^R; Km^R; Tc^R	This work
pMutin2	pBR322-based integration vector for *B. subtilis*; containing a multiple cloning site downstream of the P*spac* promoter, and a promoter-less 1998 *lacZ* gene preceded by the RBS of the *spoVG* gene; 8.6 kb; Ap^R;	Vagner et al. 1998. A vector for systematic gene inactivation in *Bacillus subtilis*. Microbiology **144**:3097-3104.